Combined Rietveld- and Stereochemical-Restraint Refinement with High-Resolution Powder Diffraction Data Offers a New Approach for Obtaining Protein-Drug Structures

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We have circumvented a major barrier to drug discovery by successfully solving protein-ligand structures directly from powder samples rather than large single crystals. With the decoding of the human genome, the paradigm of drug discovery will change to one that is focused on the creation and characterization of compounds that interact with specific proteins under a wide variety of conditions. Current techniques require either the formation of protein-ligand single crystals of sufficient quality for x-ray diffraction work or the interpretation of nuclear magnetic resonance (NMR) spectra. Growing single crystals of proteins is an arduous process, requiring careful selection of conditions to achieve success. A protein-structure study can be completely halted by the inability to produce a single crystal. Furthermore, single-crystal growth of a protein-ligand complex often presents completely new challenges compared with growing crystals of the protein alone. NMR, meanwhile, is limited by spectrometer resolution to studies of proteins with a molecular weight of no more than 25 kDa. The difficulties of studying protein-ligand complexes are best seen through a cursory examination of the Protein Data Base (http://www.rcsb.org/pdb/), which shows that only about 10% of the entries involve these complexes.

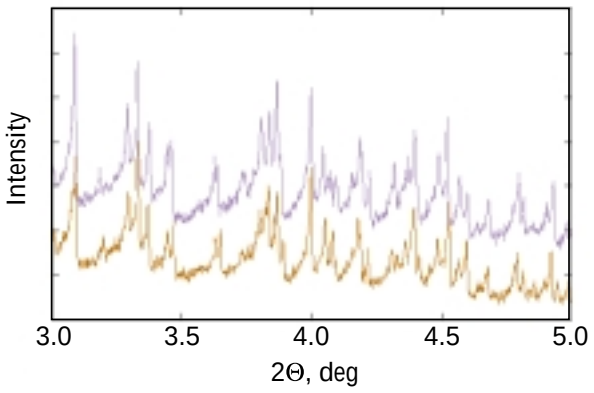
Protein Powder Diffraction

A new method for obtaining the structure of protein-drug complexes is suggested by the fact that the most easily prepared form of these materials is a polycrystalline powder consisting of many small crystals. This powder can be formed over a wide range of conditions and time scales quite unlike the restricted circumstances required for producing large, single crystals. In many cases, polycrystalline powders can be readily made, but large, single crystals prove impossible to grow. High-resolution diffraction patterns from this polycrystalline material can display considerable sensitivity to subtle structural changes typified by shifts in the diffraction peak positions and by changes in intensity. This sensitivity has been long recognized by materials scientists, and over the past 30 years considerable progress has been made in extracting structural information from powders. For example, virtually all our structural knowledge for high-temperature superconductors comes from x-ray and neutron-powder-diffraction experiments.

Superconducting materials readily form powders but are not amenable to growth as large, single crystals. Many of them are also subject to phase changes that would render single crystals useless for diffraction experiments. Powder-diffraction experiments and Rietveld refinement[1,2] have elucidated the nature of these phase changes and the very subtle structural changes that accompany changes in their superconducting properties with, for example, composition.

Until recently, protein crystal structures were considered far too complex for powder-diffraction experiments to give any useful information. However, our recent work at Brookhaven National Laboratory's National Synchrotron Light Source has shown that proteins give extremely sharp x-ray powder-diffraction patterns that can be analyzed by a combined Rietveld and stereochemical restraint refinement to give structures of moderate resolution and, in one case, has led to the first solution of a protein structure from powder-diffraction data.[3,4] Protein lattice parameters determined from these powder data are perhaps two orders of magnitude more precise than that obtained from typical single-crystal experiments.

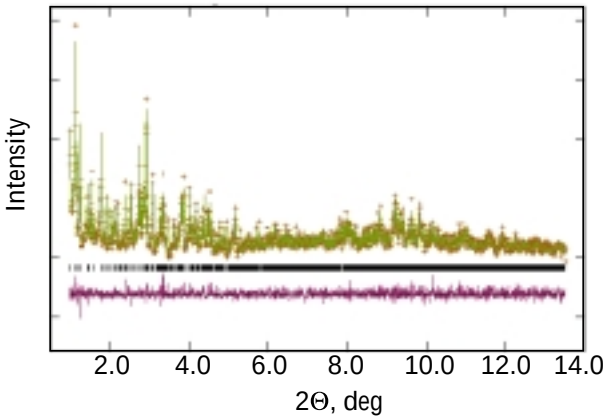
For the study of protein-ligand complexes, powder diffraction offers a distinct advantage over single-crystal work in its complete immunity to crystal fracture and to any phase change that may accompany complex formation. The extreme sensitivity of diffraction patterns to changes in lattice parameters



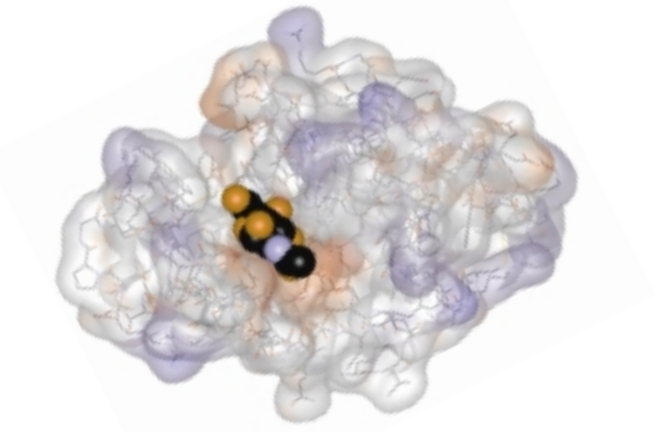
▲ ***Fig. 1.*** *A small segment of high-resolution, x-ray powder-diffraction patterns of lysozyme (red) and N-acetylglucosamine-lysozyme complex (blue), both prepared from 0.5M NaCl, 0.05M pH 6.0 buffer. The two patterns have been offset for clarity.*

makes powder diffraction sensitive to complex formation. Furthermore, rapid formation of a polycrystalline precipitate allows possible exploration of initial complex formation under a wide variety of conditions not accessible in slow-soaking or single-crystal growth experiments.

We recently explored this possibility in a study of the binding of N-acetylglucosamine (NAG) to chicken egg lysozyme.[5] In this experiment, a high-resolution powder-diffraction pattern of the protein alone was compared to that of the complex, both obtained from identical solvent mixtures (0.5M NaCl in pH 6.0/0.05M phosphate buffer). The patterns showed a clear indication (Fig. 1) of a structural change upon formation of the complex. Subsequent combined Rietveld and stereochemical restraint refinement (Fig. 2) revealed the position and orientation of the NAG ligand in the C-ligand binding site of lysozyme (Fig. 3). Interestingly, a similar comparison of materials prepared from a pH 5.0 buffer showed no indication of complex formation. This finding clearly demonstrates the usefulness of forming polycrystalline material under a wide range of conditions.



▲ ***Fig. 2.*** *High-resolution, x-ray powder diffraction profile from the final Rieveld refinement of the N-acetylglucosamine-lysozyme complex. Observed intensities are shown as red (+), calculated and difference curves as green and purple lines, and reflection positions as black (|). The background intensity found in the refinement has been subtracted from the observed and calculated intensities for clarity.*



▲ ***Fig. 3.*** *View of the molecular surface of lysozyme with a space-filling representation of the bound N-acetylglucosamine.*

Future Developments

High-resolution powder diffraction of proteins is still in its infancy, and the current molecular weight limit of perhaps 50 kDa is largely due to the density of reflection overlaps in the diffraction pattern. These limits are stricter than those of single-crystal diffraction, and there is no present way of solving protein structures *ab initio* from powder data; but model building and molecular replacement work quite well. Nonetheless, we can easily see future developments of the method that will allow examination of protein structures that exceed 100 kDa.

In particular, current data-collection technology scans the powder-diffraction pattern a few points at a time over a narrow field of view. Consequently, data-collection times at a synchrotron source are on the order of half a day. The use of high-resolution imaging technology and x-ray focusing optics should improve this by a thousand-fold or more, making it possible to use powder diffraction on a laboratory x-ray source to screen for the formation of protein-drug complexes and to determine their structures.

References

1. H. M. Rietveld, "A Profile Refinement Method for Nuclear and Magnetic Structures," *Journal of Applied Crystallography* **2**, 65-71 (1969).
2. The Rietveld Method (Young, R. A., Ed.), (Oxford University Press, New York, 1993).
3. R. B. Von Dreele, "Combined Rietveld and Stereochemical Restraint Refinement of a Protein Crystal Structure," *Journal of Applied Crystallography* **32**, 1084-1089 (1999).
4. R. B. Von Dreele *et al.*, "The First Protein Crystal Structure Determined from High Resolution X-Ray Powder Diffraction Data: A Variant of T3R3 Human Insulin Zinc Complex Produced by Grinding," *Acta Crystallographica D* **56**, 1549-1553 (2000).
5. R. B. Von Dreele, "Binding of N-acetylglucosamine to Chicken Egg Lysozyme: A Powder Diffraction Study," *Acta Crystallographica D* (submitted).

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